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GLUCOSAMINE SULFATE INHIBITS IL-1-STIMULATED GENE EXPRESSION AT CONCENTRATIONS FOUND IN HUMAN PLASMA AFTER ORAL INTAKE

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Aim of Study: In-vitro studies investigating the mechanism of action of glucosamine sulfate in osteoarthritis have been mostly performed at high glucosamine concentrations. Recent studies in man have shown that after administration of the standard 1500 mg once-a-day oral therapeutic dose of glucosamine sulfate for osteoarthritis, average maximum plasma concentrations of glucosamine are in the 10 μM range. The aim of the present study was to investigate in a human chondrocyte-like cell model, the effects of glucosamine sulfate at these concentrations on IL-1-induced gene expression of different inflammation or matrix degradation markers. In addition, the effect of 10 μM glucosamine sulfate was studied on P-IkB levels in the cytoplasm. IkB is the inhibitory protein that maintains the transcription factor NFkB in an inactivated state: when phosphorilated, it is released from NF-kB that can thus enter the nucleus.
Methods: Human chondrosarcoma cells SW 1353 were grown to confluence and stimulated with 2 ng/ml IL-1β, i.e. the optimal stimulatory concentration in pilot experiments. Cells were incubated in drug-free medium or containing glucosamine sulfate between 0.001 and 100 μM. RealTime PCR was used to quantify gene expression levels after optimal exposure, that was 6 hours for Cox-2, iNOS, IL-1β, IL-6 and MMP-3, 1 hour for TNFα and 24 hours for ADAMTS5 (aggrecanase-2). All experiments were run in triplicate. Results were expressed as glucosamine concentrations able to decrease by 50% (IC50) the stimulation by IL-1β. P-IkB levels were determined by Western Blot: cell lysates of SW 1353 stimulated for 45 min with 2 ng/ml IL-1β were analysed by SDS-PAGE, detection being performed using ECL (Amersham) and quantitation by densitometry analysis using NIH Image software.
Results: Glucosamine sulfate decreased the strongly IL-1β-stimulated gene expression of all transcripts considered, with glucosamine IC50 close to 10 μM or lower (Table 1).

Table 1. Glucosamine IC 50 (μM ± SE) on IL-1b-stimulated gene expression of different markers

Cox-2	iNOS	IL-1 b	IL-6	MMP-3	TNF-a	ADAMTS5
11.2±1.2	13.8±5.6	6.2±3.0	4.4±1.1	10.2±2.3	12.8±2.0	2.8±0.7

IL-1β stimulation induced a strong activation of NFkB and therefore a parallel 4-fold increase in P-IkB cytoplasmic levels. Glucosamine sulfate inhibited this increase by 93% at the concentration used.
Conclusions: Glucosamine sulfate inhibits IL-1-stimulated gene expression of different inflammation or matrix degradation markers in a human chondrocyte-like cell model, at glucosamine concentrations found in human plasma after oral therapeutic doses. These results confirm previous observations obtained with higher glucosamine concentrations and that these events are probably initiated by a decrease in NFkB activation and nuclear translocation.

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OA JOINT FLUID IS DEFICIENT IN CRITICAL ANTIOXIDANTS

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Aim of Study: To determine if extracellular superoxide dismutase (EC-SOD) in human joint fluid reflects the deficiency in this enzyme found in OA cartilage.
Methods: Human joint fluid was collected from patients undergoing arthrocentesis for diagnosis or joint injection (OA patients, n=40) or from patients undergoing arthroscopy for meniscal tears or ACL reconstruction (Controls, n=12). Superoxide dismutase (SOD) activity was measured by cytochrome C assay. The extracellular SOD isoenzyme protein was determined with an ELISA. Urate and ascorbate were measured using HPLC. Reduced and oxidized glutathione (GSH/GSSG) were measured with a colorimetic technique (OxisResearch) and TGF beta and IL-6 were measured with ELISA. Total antioxidant power was also measured with a colorimetic reduction of copper (Oxford) and nitrates/nitrites were measured with the Griess reaction. Western blots were done for EC-SOD to assess the percentage of the protein with and without it's heparin binding site and compared to cartilage tissue extracts.
Results: The major catalytic antioxidant found in human joint fluid is extracellular superoxide dismutase (EC-SOD) and it was decreased fifty percent in the OA joint fluid samples (*p=0.003*). Western blots showed that in joint fluid the extracellular matrix (ECM)-binding site of EC-SOD is cleaved in 70% of the molecules. In cartilage tissue from controls only about 25% of EC-SOD molecules have the binding site cleaved. The relative percent of cleaved enzyme in OA joint fluid is greater than in control joint fluid. The low molecular weight antioxidants: ascorbate (p=0.02), oxidized glutathione (p=0.002), and reduced glutathione (0.001) were significantly decreased in the OA samples.
Conclusions: We find that there are marked decreases in both enzymatic (EC-SOD) and low molecular weight antioxidants in OA joint fluid. We have previously shown that EC-SOD is profoundly decreased in both human OA cartilage and in a mouse model of OA (STR/ort mouse). In this model there is an inadequate EC-SOD response to high oxidant stress levels. Osteoarthritis is associated with increased production of inflammatory cytokines and ultimate mechanical failure of the articular cartilage ECM. Excess oxidants lead to activation of MMP's and damage to the structural molecules of the ECM, that exacerbates the vulnerability of the tissue to mechanical damage. The finding of decreases in critical joint fluid extracellular antioxidants further supports the observation that oxidant damage plays an important role in the pathogenesis of OA. These results in joint fluid suggest that there is an inadequate response to oxidant stress in patients with symptomatic disease. Loss of adequate oxidant scavenging from the joint fluid may intensify or initiate the oxidant stress found in OA cartilage. Measuring joint fluid antioxidants may be a sensitive test for identifying early arthritis.